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Original contribution

Clinicopathologic significance of immunostaining of α-thalassemia/mental retardation syndrome X-linked protein and death domain–associated protein in neuroendocrine tumors

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Summary α-Thalassemia/mental retardation syndrome X-linked protein (*ATRX*) and death domain–associated protein (*DAXX*) genes are tumor suppressors whose mutations have been identified in sporadic pancreatic neuroendocrine tumors as well as in patients with MEN1. However, it is unknown whether *ATRX* and *DAXX* alterations are specific for pancreatic neuroendocrine tumor. In addition, the association of ATRX/DAXX protein loss with tumor cell proliferation has not been examined. We, therefore, immunostained ATRX and DAXX in 10 gastric, 15 duodenal, 20 rectal, 70 pancreatic, and 22 pulmonary neuroendocrine tumors with 15 nonneoplastic pancreases and 27 pancreatic adenocarcinomas to elucidate the site-specific roles of ATRX/DAXX abnormalities. At least 1 loss of ATRX and DAXX immunoreactivity was detected in all neuroendocrine tumor cases but not in any of nonneoplastic pancreatic tissues or pancreatic adenocarcinomas. The loss of DAXX protein was correlated with the Ki-67 index (ATRX, $P = .904$; DAXX, $P = .044$). The status of DAXX immunoreactivity correlated positively with World Health Organization histologic grade ($P = .026$). These results suggest that the status of ATRX or DAXX protein loss in neuroendocrine tumor differed among the organs in which these tumors arose, and these proteins may play site-specific roles in the development of these tumors.

1. Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms arising from neuroendocrine cell compartments in various organs. Despite marked diversity in the tissues of

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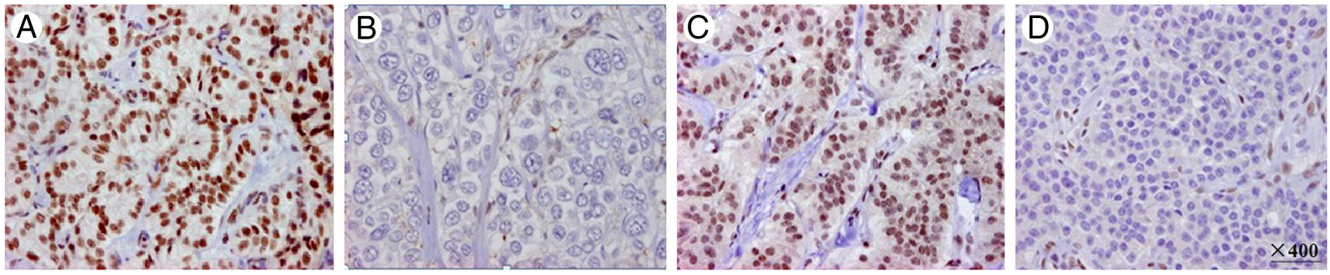

Fig. 1 Immunohistochemical expression of ATRX and DAXX protein in PNETs. Nuclear immunolabeling of ATRX (A) and DAXX (C). Loss of nuclear expression of ATRX (B) and DAXX (D).

origin, these tumors share certain features, that is, the production and secretion of amines or polypeptide hormones or both [1]. Approximately two-thirds of NETs occur in the gastrointestinal tract, one quarter in the bronchopulmonary system, and the rest in other endocrine tissues such as the thyroid or in nonendocrine organs [2,3]. The results of an epidemiologic study of NET patients in the United States demonstrated that the primary tumor site could determine the prognosis. For instance, rectal NET was associated with a relatively favorable prognosis, whereas pancreatic NET had the worst survival time [4]. In addition, a significant difference exists between Japanese and Western patients in terms of tumor frequency and location, but the prognostic values according to the disease location are also applicable to Japanese patients [5].

Relatively complicated intracellular signaling pathways play important roles in the growth of NETs, and their prognostic or therapeutic values, therefore, have been studied extensively [6]. Among the factors involved in these pathways, Jiao et al [7] recently reported that mutations were present in the α-thalassemia/mental retardation syndrome X-linked protein (*ATRX*) and death domain–associated protein (*DAXX*) genes, which were detected in 18% and 25%, respectively, of pancreatic NETs (PNETs). In addition, mutations of these 2 genes were associated with the loss of expression of their corresponding proteins [7].

ATRX is a member of the SNF2 family of ATP-dependent chromatin-remodeling proteins [8-10]. DAXX is a multifunctional nuclear protein that modulates both apoptosis and transcription and also interacts with a number of transcription factors [8]. Both ATRX and DAXX form stable protein complexes [8] and are co-located with pericentric heterochromatin and promyelocytic leukemia bodies [11,12]. In addition, the ATRX/DAXX protein complex participates in chromatin remodeling and can assist DAXX in the assembly of H3.3 nucleosomes [11].

Mutations of *ATRX* and *DAXX* have been reported in sporadic PNETs, but it remains unknown whether these 2 genes are altered in NETs of other organs. The loss of expression of these 2 genes has been associated with the size of PNETs, but their association with tumor proliferative activity, which constitutes the basis of the recently defined World Health Organization (WHO) histopathologic classification [13], remains unknown. Therefore, in this study, we stained ATRX/DAXX in NETs of the pancreas and nonpancreatic organs histochemically to elucidate the clinicopathologic significance of these 2 proteins.

2. Materials and methods

2.1. Patients

All 137 tumor samples originating from Japanese patients with primary NET who underwent surgical resection from 1992 to 2010 at Tohoku University Hospital, Sendai, Japan, were evaluated. These specimens consisted of 10 gastric, 15 duodenal, 20 rectal, 70 pancreatic, and 22 pulmonary NETs. Of the patients, 74 were female and 63 were male, and the median age was 56 years (range, 12-86 years). In addition, 15 nonneoplastic pancreatic tissue and 27 pancreatic adenocarcinomas were evaluated. The clinicopathologic features examined were tumor size, lymph node involvement, and distant metastasis

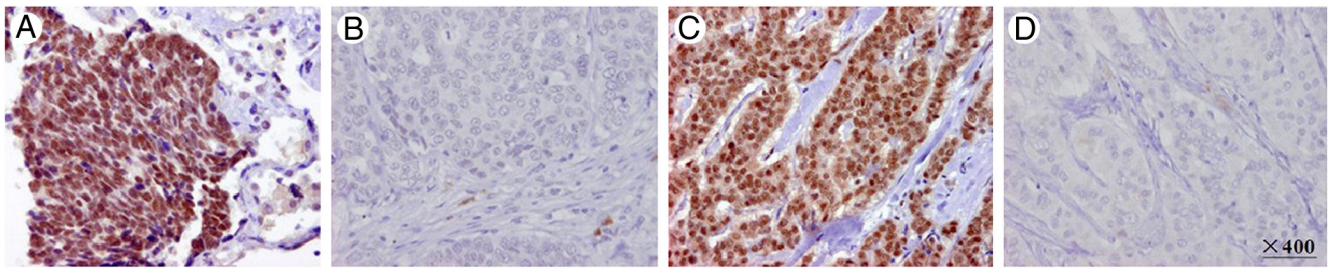

Fig. 2 Immunohistochemical expression of ATRX and DAXX in nonpancreatic NET. Positive nuclear immunolabeling (A) and loss of nuclear protein expression (B) of ATRX in a pulmonary NET. Similarly, positive nuclear immunolabeling (C) and loss of nuclear protein expression (D) of DAXX in a rectal NET.